

Blasticidin S HCl

Using Blasticidin

Description

Blasticidin S HCl is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes* which inhibits protein synthesis in both prokaryotic and eukaryotic cells (Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965). Resistance is conferred by expression of either one of two blasticidin S deaminase genes: *BSD* from *Aspergillus terreus* (Kimura *et al.*, 1994) or *bsr* from *Bacillus cereus* (Izumi *et al.*, 1991). These deaminases convert blasticidin S to a non-toxic deaminohydroxy derivative (Izumi *et al.*, 1991).

Contents and Storage

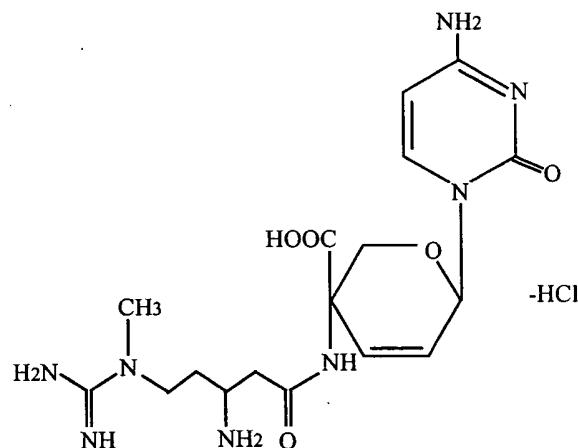
50 mg of Blasticidin S HCl is supplied as white, solid powder and shipped at room temperature. Upon receipt, store the powder at +4°C. Product is stable for two years as supplied.

Molecular Weight, Formula, and Structure

Merck Index: 12: 1350

MW: 458.9

Formula: C₁₇H₂₆N₈O₅-HCl



Handling Blasticidin

Always wear gloves, mask, goggles, and protective clothing (e.g. a laboratory coat) when handling blasticidin. Weigh out blasticidin and prepare solutions in a hood.

Preparing and Storing Stock Solutions

Blasticidin is soluble in water and acetic acid. Sterile water is generally used to prepare stock solutions of 5 to 10 mg/ml.

- Dissolve blasticidin S in sterile water and filter-sterilize the solution.
- Aliquot in small volumes suitable for one time use (see next to last point below) and freeze at -20°C for long-term storage or store at +4°C for short term storage.
- Aqueous stock solutions are stable for 1-2 weeks at +4°C and 6-8 weeks at -20°C.
- pH of the aqueous solution should not exceed 7 to prevent inactivation of blasticidin.
- Do not subject stock solutions to freeze/thaw cycles (**do not store in a frost-free freezer**).
- Upon thawing, use what you need and discard the unused portion.
- Medium containing blasticidin may be stored at +4°C for up to 2 weeks.

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Using Blasticidin, continued

Selection in *E. coli*

For selection of blasticidin-resistant *E. coli*, use Low Salt LB medium containing 50-100 µg/ml blasticidin (see recipe below). Depending on the bacterial strain that you use, you may have to optimize the blasticidin concentration. If you get a lawn of bacteria on your Low Salt LB plate instead of individual bacterial colonies then increase the blasticidin concentration to 100 µg/ml in the plate.

Please note that the salt concentration of the medium must remain low (<90 mM) and the pH should not exceed 7.0. Failure to lower the salt content of your LB medium will result in non-selection due to inhibition of the drug unless a higher concentration of blasticidin is used.

Low Salt LB Medium

10 g Tryptone

5 g NaCl

5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 950 ml. Adjust pH to 7.0 with 1 N NaOH. Bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle at 15 psi and 121°C for 20 minutes.
3. Allow the medium to cool to at least 55°C before adding the blasticidin to 50 µg/ml final concentration.
4. Store plates at +4°C in the dark. Plates with blasticidin are stable for up to 2 weeks..

Selection in Yeast

The concentration of blasticidin required for selection in yeast can vary substantially depending on the particular species, strain, and type of medium used. Generally, concentrations for selection range from 25 to 300 µg/ml blasticidin. We recommend that you perform a kill curve for each species, strain, and medium combination to determine the appropriate concentration of blasticidin to use for selection of resistant cells.

Selection in Mammalian Cell Lines

Typically, concentrations between 2 and 10 µg/ml blasticidin are sufficient for selection in mammalian cells. Be sure to determine the concentration sufficient to kill untransfected cells for each cell line you use.

References

- Izumi, M., Miyazawa, H., Kamakura, T., Yamaguchi, I., Endo, T., and Hanaoka, F. (1991). Blasticidin S-Resistance Gene (*bsr*): A Novel Selectable Marker for Mammalian Cells. *Exp. Cell Res.* 197, 229-233.
- Kimura, M., Takatsuki, A., and Yamaguchi, I. (1994). Blasticidin S Deaminase Gene from *Aspergillus terreus* (*BSD*): A New Drug Resistance Gene for Transfection of Mammalian Cells. *Biochim. Biophys. Acta* 1219, 653-659.
- Takeuchi, S., Hirayama, K., Ueda, K., Sakai, H., and Yonehara, H. (1958). Blasticidin S, A New Antibiotic. *The Journal of Antibiotics, Series A* 11, 1-5.
- Yamaguchi, H., Yamamoto, C., and Tanaka, N. (1965). Inhibition of Protein Synthesis by Blasticidin S. I. Studies with Cell-free Systems from Bacterial and Mammalian Cells. *J. Biochem. (Tokyo)* 57, 667-677.